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EFFECTS OF SIMULATED SPACE ENVIRONMENTS ON THE VIABILITY OF MICROORGANISMS

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INTRODUCTION

This report under Contract No. NASr-41, "Effects of Simulated Space Environment on the Viability of Microorganisms," National Aeronautics and Space Administration, Washington, D. C., summarizes the results obtained during the period April 16 through July 15, 1962. The experimental program was a joint effort of National Research Corporation, and the Department of Nutrition, Food Science and Technology, Massachusetts Institute of Technology, Cambridge, Massachusetts. The previous technical report was dated June 22, 1962, and considered the work accomplished during the period January 16 through April 15, 1962. All manipulative procedures were detailed in the last report (Davis et al., 1962) and will not be described here except where clarification is necessary.

We have completed some experiments in which dry spores were heated for several days at particular temperatures while at atmospheric pressure and have compared the survival data with the results of the temperature-vacuum experiments described previously (Silverman, 1962). The results of some preliminary experiments on the survival of spores irradiated with gamma rays while in vacuum will also be presented in this report.

TEMPERATURE EXPERIMENTS

In the preceding report it was shown that Bacillus subtilis var. niger was the only one of four organisms which withstood one day at 90°C and atmospheric pressure. In fact, spores of this organism were still recovered after 7 days at this temperature. B. megaterium has since been tested in the same manner, and yielded 0.005 per cent survival at 24 hours. No survivors could be detected at 48 hours. The results have been reproduced in entirety in Table 1.

Although ultrahigh vacuum at ambient temperature (ca. 25°C) is, in general, not injurious to the spores under study, the previous report showed that B. subtilis var. niger and Aspergillus niger were considerably more resistant to moderate temperatures than the other three organisms. The influence of vacuum in modifying survival can be demonstrated in Table 2. The survival data from vacuum and atmospheric pressure experiments at 25°C shows that survival is ordinarily not markedly different at both conditions for each organism. On the other hand, at 60°C one may observe significant difference in survival at the two conditions of pressure. Aside from the temperature effect on survival, a vacuum effect is indicated.

It is probable that each organism would have a temperature range over which the vacuum effect would be especially pronounced. The tenfold differences in initial spore numbers in the atmospheric pressure and vacuum experiments probably do not account for the differences in recovery. The resistance of B. subtilis var. niger at 60°C raises the possibility that this organism would show the vacuum effect at a higher temperature. At 90°C there were no survivors in vacuum with 4.4×10^7

TABLE 1

SURVIVAL OF SPORES AT 90°C AND ATMOSPHERIC PRESSURE

Organism	Number of Days Exposure						
	1 day %	2 days %	3 days %	4 days %	5 days %	6 days %	7 days %
B. stearothermophilus	0	---	---	---	---	---	---
B. subtilis var. niger (Expt. A)	48	26.5	11	3.1	0.55	0.08	0.002
B. subtilis var. niger (Expt. B)	32.6	12.6	4.2	0.46	0.09	---	---
B. megaterium	0.005	0	---	---	---	---	---
C. sporogenes	0	---	---	---	---	---	---
A. niger	0	---	---	---	---	---	---

TABLE 2

PER CENT SURVIVAL OF DRY SPORES DURING FIVE DAYS AT 25°C AND 60°C IN VACUUM AND AT ATMOSPHERIC PRESSURE

Organism	Atmospheric Pressure				Ultrahigh Vacuum		
	Days at 60°C			Days at 25°C	Days at 60°C		Days at 25°C
	0	4	5	5	0	4-5	4-5
	spores/filter	%	%	%	spores/filter	%	%
B. stearothermophilus	1.6×10^6	30	35	56	1.3×10^5	1.2	67
B. subtilis var. niger	1.8×10^6	67	72	78	1.8×10^5	40	113
B. megaterium	1.5×10^6	80	87	87	1.8×10^5	0.7	98
C. sporogenes	1.6×10^6	48	62	24[1]	3.0×10^5	0.1	88
A. niger	0.7×10^6	61	9	120	1.3×10^5	25	98

[1] Duplicate filters yielded low counts.

spores while at atmospheric pressure 0.9×10^4 -- 7×10^4 spores were recovered from 2×10^6 present initially.

RADIATION EXPERIMENTS

Some organisms are more sensitive to ionizing radiation when they are irradiated in air than when they receive the same radiation dose under anoxic conditions. This oxygen effect is known to occur in dry spores of B. megaterium (Powers et al., 1959). The magnitude of the oxygen effect is less in dry spores than in spores in water suspension. Powers' group observed that the oxygen effect was manifested over a temperature range from -148°C to +40°C, but vanished below -150°C. Ebert (1960) indicated that the decrease of the oxygen effect with decreasing temperature might be related to changes occurring in the residual water of the spores. Powers and his associates (1960, 1961) investigated the inactivation of dry bacterial spores as a function of temperature during and following ionizing irradiation. Dried B. megaterium spores increased in radiation sensitivity as the temperature during irradiation in nitrogen rose over the range -150°C to +30°C. The maximum at 30°C was about 30 per cent above the response at low temperature. In the presence of oxygen the radiation sensitivity increase occurred at a higher rate (25 per cent greater at 30°C in oxygen than in nitrogen) and continued to increase as the temperature was raised above 30°C. Under anoxic conditions above 30°C, sensitivity decreased to a minimum at 80°C. The spores were half as sensitive at this temperature as they were at 30°C, and considerably less sensitive than at extremely low temperature.

A postirradiation oxygen effect which was independent of the temperature during irradiation was also demonstrated. Heat (80°C, 20 minutes) in the absence of oxygen halved the radiation effect. Nitric oxide, a free radical scavenger, protected the spores in the same fashion as the

postirradiation heat treatment. Powers' group concluded that free radicals reacting with oxygen become irreversibly toxic to the cells, while heat treatment removes the free radicals. Under anoxic conditions the free radicals are considered to be slowly fixed in the lethal state.

Pepper, Buffa, and Chandler (1956) found that 0.6 -- 2.1 megareps cathode rays were necessary to sterilize filter paper discs impregnated with 5×10^4 spores of the 25 bacteria tested. Only Clostridium tetani and B. pumilis required 2.1 megareps. They noted that B. pumilis spores irradiated in the dry state were more sensitive than spores irradiated while frozen.

Although Escherichia coli exhibits a greatly increased radiosensitivity in the presence of oxygen, Bellamy et al. (1955) pointed out that spores of B. subtilis are much less sensitive, and spores of B. thermoacidurans are practially insensitive to oxygen during irradiation.

Newell and Naugle (1960) observed that radiation effects due to the electromagnetic radiations present in outer space are expected to be of little significance. They presumed that x-rays and gamma rays produced by the interaction of charged particles with the spacecraft itself would consitute the principle hazard from electromagnetic radiation. Considering the lack of information on the effect of the space environment on microorganisms, it would appear that cosmic gamma radiation would not directly insure the destruction of microorganisms on the surface of an interplanetary vehicle. Sagan (1961) has in fact suggested that microorganisms having mean lethal doses as high as 10^7 rep might exist although no systematic search for such radioresistant organisms has been undertaken.

Materials and Methods

A glass manifold (Figures 1 and 2) was constructed so that spores could be exposed to ultrahigh vacuum for several days at NRC and then sealed off in tubes while under vacuum. The tubes were then irradiated at MIT in the Mk I Co^{60} Irradiator at 5500 rads per minute.

The manifold was fabricated from 2-inch diameter Pyrex glass and had an overall length of 27.5 inches. A 10-inch extension of 2-inch stainless steel tubing was fused to the glass by graded seals, and the stainless steel section was welded to a 3-cubic foot ultrahigh vacuum chamber. Seven glass tubes (28 x 300 mm.) were sealed to the manifold on 4-inch centers. The first tube was attached 6 inches from the glass-metal seal. The tubes were prepared for subsequent sealing by constricting and thickening the neck about 3.5 inches from the manifold. A short accessory tube fused to the bottom of each manifold tube was to be cut off at the time the filters within the tube were removed for plating in order to return the tube to atmospheric pressure with minimum disturbance to the specimens.

Pressures were measured at two positions on the manifold and at a glass side arm on the ultrahigh vacuum chamber by the Nottingham modification of the Bayard-Alpert ultrahigh vacuum gauge. Pressures were recorded on a multi-channel recorder, and were noted at the time each tube was sealed off. The complete system was tested for leaks by pumping for several days before the spores were placed in the tubes.

Radiation resistivity of spores of B. stearothermophilus, B. subtilis var. niger, B. megaterium, C. sporogenes, and A. niger was studied in in these experiments. Spores were placed on glass fiber filters (ca. 10^6 spores/filter) as described previously (Davis et al., 1962). The filters

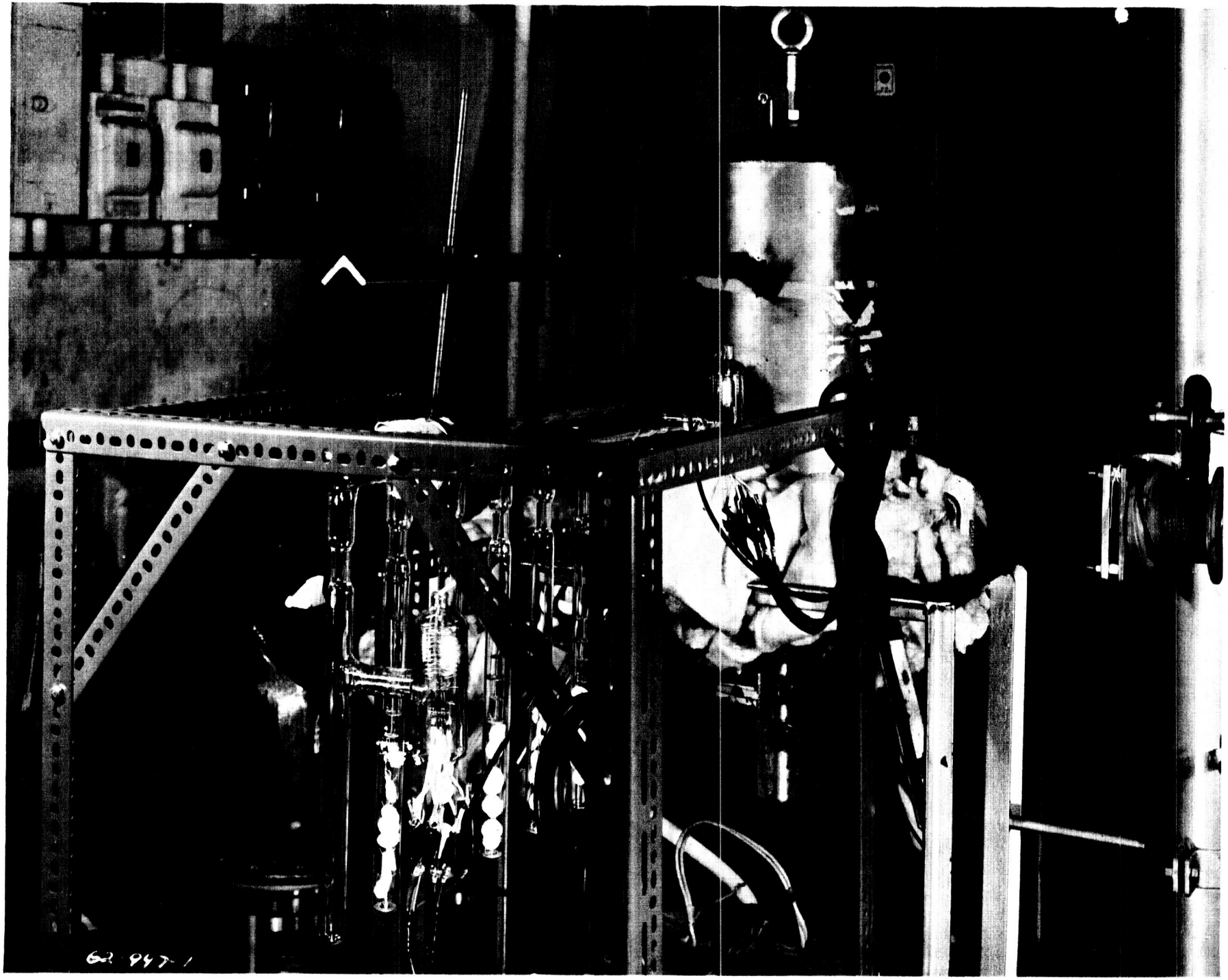

FIGURE 1 -- THE GLASS MANIFOLD-ULTRAHIGH VACUUM CHAMBER SYSTEM

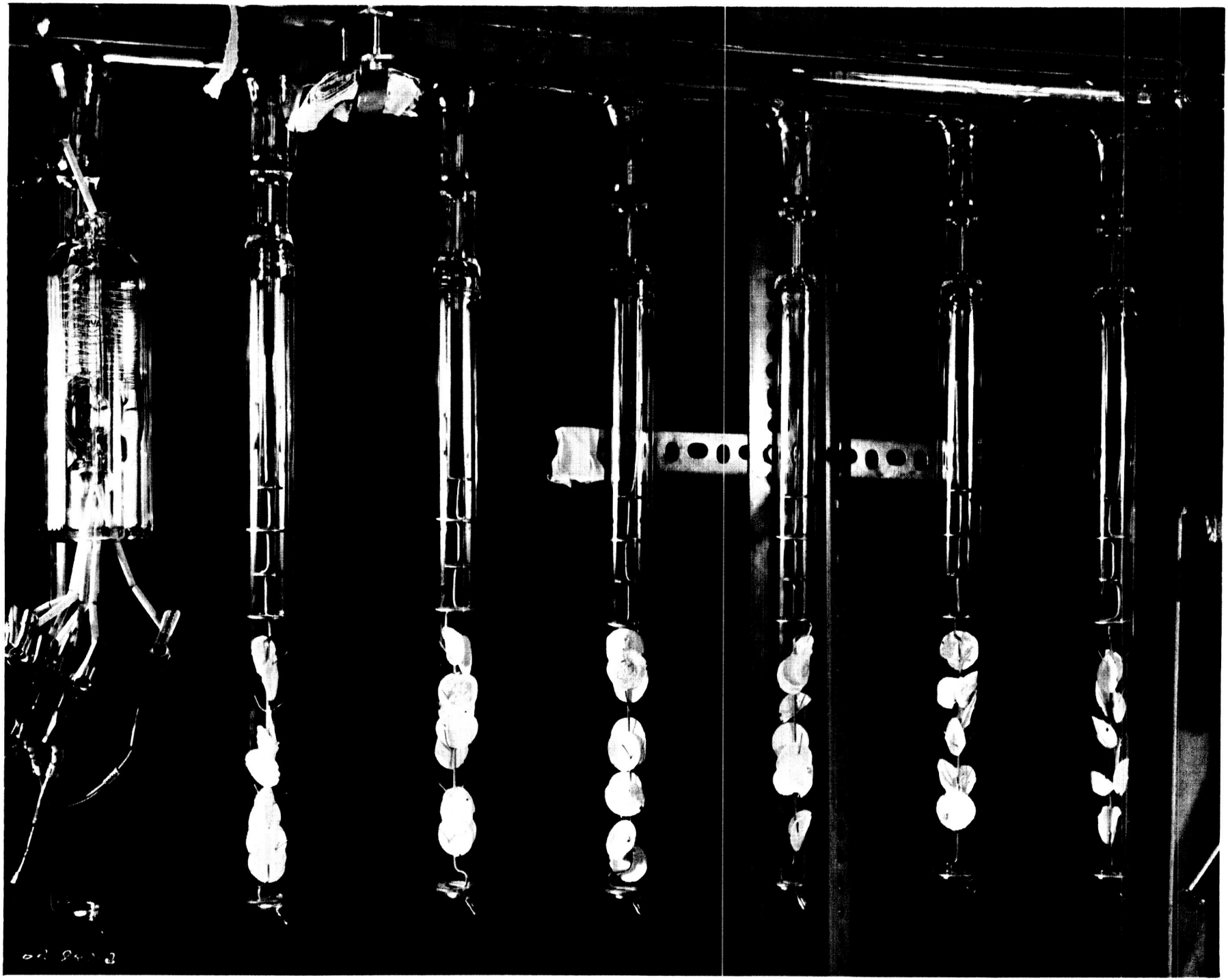

FIGURE 2 -- THE MANIFOLD AND SPORES DURING PUMPDOWN

were mounted on stainless steel wire supports so that they would be suspended vertically when the supports were positioned in the tubes. Two filters impregnated with each of the five organisms were placed on each support.

When the system leak test was completed, six of the tubes were cut off, the spore supports inserted, and the tubes resealed to the manifold. A series of metal discs was welded to the upper part of the supports to protect spores from radiant heat during sealing. The system was evacuated for 5 days after which the six tubes containing spores were sealed off under vacuum and separated from the manifold.

The following experiments were performed during a two-week period with spores on filters sealed in tubes under vacuum and with control filters stored in a silica gel desiccator:

A: Tube No. 1 irradiated to 200,000 rads; unirradiated desiccator controls.

B: Tube No. 2 irradiated to 100,000 rads; Tube No. 3 plated as a vacuum control.

C: Tube No. 4 irradiated to 200,000 rads; filters from desiccator irradiated to 200,000 rads; unirradiated desiccator controls.

D: Tube No. 5 opened to admit air immediately before irradiation to 200,000 rads; Tube No. 6 irradiated to 100,000 rads; filters from desiccator irradiated to 200,000 rads.

The plating procedure for the five organisms was described in the previous report.

Results and Discussion

The pressure drop in the vacuum system was somewhat slower than anticipated, presumably because of outgassing from the glass. After 3 days, The system pressure (at the chamber) was 1.2×10^{-9} torr while the two gauges on the manifold indicated 4.6×10^{-8} torr and 3.4×10^{-8} torr. The following day the chamber was at 4×10^{-10} torr and the manifold gauges registered 1--2 $\times 10^{-8}$ torr.

Some pressure rise was experienced when the tubes were sealed off with a torch at the conclusion of the evacuation period. Outgassing from the heated glass undoubtedly contributed to this pressure rise. It may be seen from Table 3 that although the spores had been in ultrahigh vacuum, a sharp pressure rise occurred when the tubes were sealed off. Nevertheless, subsequent irradiation experiments could be related to the extremely dry state of the spores. It must be stressed that these results are to be considered preliminary and subject to change since the apparatus and experimental approach will be modified to strengthen the reliability and precision of the data.

These initial radiation experiments have been summarized in Tables 4, 5, and 6. The results of the four experimental periods have been segregated in Table 4 so that data obtained from each experiment is on the same horizontal line. Note that several experiments were done twice. The data were reduced to per cent survival values (Tables 5 and 6) to facilitate interpretation of the results and to take into account the stability of the spores in vacuum and air.

Unirradiated controls in vacuum and in air were compared in Table 5 (Column C) to determine the role of vacuum in reducing the number of

TABLE 3

PRESSURES AT THE TIME TUBES WERE SEALED OFF

AND REMOVED FROM THE MANIFOLD

Tube	System Pressure (torr)	Proximal Gauge (torr)	Distal Gauge (torr)
1	2.1×10^{-9}	2.0×10^{-7}	1.7×10^{-7}
2	2.5×10^{-9}	3.0×10^{-7}	2.7×10^{-7}
3	1.8×10^{-9}	1.7×10^{-7}	1.8×10^{-7}
4	2.8×10^{-9}	3.0×10^{-7}	3.8×10^{-7}
5	1.5×10^{-9}	7.3×10^{-8}	1.3×10^{-7}
6	1.7×10^{-9}	6.4×10^{-8}	1.3×10^{-7}

TABLE 4

SURVIVAL OF SPORES FOLLOWING GAMMA IRRADIATION

IN VACUUM AND AT ATMOSPHERIC PRESSURE

Organism	Expt.[1]	A 100,000 rads Vacuum	B 100,000 rads Air (desiccator)	C 200,000 rads Vacuum	D 200,000 rads Opened Tube[2]	E 200,000 rads Air (desiccator)	F Unirradiated Vacuum	G Unirradiated Air (desiccator)
B. stearothermophilus	A			4.1×10^4			6.7×10^5	1.8×10^6
	B	6.3×10^5	6.9×10^5	1.1×10^4		2.7×10^4		1.2×10^6
	C				4.0×10^3	3.0×10^4		
	D	4.7×10^5						
B. subtilis var. niger	A			3.9×10^4			6.0×10^5	1.7×10^6
	B	4.1×10^5	3.3×10^5	3.1×10^4		1.1×10^4		1.4×10^6
	C				9.3×10^3	1.9×10^4		
	D	1.5×10^5						
B. megaterium	A			4.9×10^5			1.9×10^6	1.7×10^6
	B	8.0×10^4	1.4×10^6	4.5×10^5		5.0×10^5		1.2×10^6
	C				1.0×10^5	6.2×10^5		
	D	7.1×10^5						
C. sporogenes	A			1.2×10^4			1.1×10^5	1.2×10^6
	B	1.0×10^5	5.3×10^5	2.2×10^3		1.5×10^5		1.0×10^6
	C				1.3×10^3	2.0×10^5		
	D	4.8×10^4						
A. niger	A			1.1×10^3			8.0×10^5	3.3×10^5
	B	1.4×10^5	9.2×10^2	1.0×10^3		7.0×10^1		1.3×10^6
	C				4.2×10^2	0		
	D	1.9×10^5						

[1]Date Performed: A: 5/23/62 C: 5/31/62
B: 5/24/62 D: 6/6/62

[2]Evacuated tube #5, opened immediately before irradiation.

TABLE 5

PER CENT SURVIVAL OF SPORES IRRADIATED TO 100,000 RADS

Organism	A[1] IV(100)/UV	B IA(100)/UA	C UV(100)/UA	D[3] UV(100)/UA (Previous Data)
B. stearothermophilus	94[2] 70	46	45	67
B. subtilis var. niger	68 25	26	38	113
B. megaterium	4.2 37	93	127	98
C. sporogenes	91 44	48	10	88
A. niger	18 24	0.1	98	98

[1]Symbols: I, irradiated; U, unirradiated; V, vacuum; A, air.

[2]Data from two experiments were calculated where two figures appear.

[3]Data from ambient temperature experiments in which filters were supported on shelves in the vacuum chamber.

TABLE 6

PER CENT SURVIVAL OF SPORES IRRADIATED TO 200,000 RADS

Organism	A[1] $\frac{IV(100)}{UV}$	B $\frac{IA(100)}{UA}$	C[3] $\frac{IV_{opened}(100)}{UV}$	D[3] $\frac{IV_{opened}(100)}{UV}$
B. stearothermophilus	6.1[2] 1.6	1.9	0.6	15
B. subtilis var. niger	6.5 5.2	0.9	1.6	27
B. megaterium	26 24	37	5.3	21
C. sporogenes	11 2.0	16.4	1.2	18
A. niger	0.14 0.13	0.004	0.05	38

[1]Symbols: I, irradiated; U, unirradiated; V, vacuum; A, air.

[2]Data from two experiments were calculated where two figures appear.

[3]Evacuated tube #5, opened immediately before irradiation.

viable spores subsequently exposed to gamma irradiation. Survivors from irradiated evacuated tubes were compared with the spores remaining after vacuum treatment and not with the initial number of spores placed on the filters. Survival data can be correlated only for the five organisms grouped within each irradiation condition of vacuum or air, unless as with B. megaterium and A. niger, vacuum and air controls were not significantly different.

These results would suggest that about half the B. stearothermophilus and B. subtilis var. niger spores were killed or otherwise lost during pumpdown. The rather low recovery of B. subtilis var. niger (30%) and C. sporogenes (10%) does not agree with previous vacuum experiments (Column D, Table 5) which indicated ambient temperature survival of 113 and 88 per cent respectively. The 127 per cent survival of B. megaterium probably reflects experimental error but could be in agreement with the insensitivity of the organism to vacuum that was observed in earlier work. It must be emphasized that these experiments will be repeated.

One may observe that B. stearothermophilus spores which survived vacuum (about half the initial spores) are quite resistant to 100,000 rads. On the other hand, although essentially all A. niger spores withstood vacuum, those spores were less tolerant of 100,000 rads than any of the bacterial spores. The extremely dry A. niger spores in vacuum were about 20 times more resistant to radiation than "dry" spores irradiated in air. At 200,000 rads (Table 6), A. niger spores were about 35 times more resistant in vacuum than in air. The small number of spores involved in these calculations (Table 4) certainly influences the reliability of these conclusions.

A dose of 100,000 rads is ordinarily of little consequence to bacterial spores. It appears that all but B. megaterium decreased exponentially with an increase in radiation to 200,000 rads. The apparently greater resistance of B. megaterium is probably attributable to the sigmoidal survival curve (multiple target) response of this organism to ionizing radiation (Woese, 1959).

C. sporogenes irradiated in air (Column B, Table 6) was not as sensitive as B. stearothermophilus, B. subtilis var. niger, and A. niger. Loss of 90 per cent of the spores in vacuum, however, resulted in a significantly smaller net recovery of C. sporogenes spores irradiated in vacuum than in air (Column C and E, Table 4). The ultimate recovery obtained for the other bacteria did not indicate any appreciable differences in air and vacuum in spite of the variation in sensitivity to vacuum and to irradiation in air and vacuum.

The situation with respect to the evacuated tube opened immediately before irradiation is of interest since loss of 62--85 per cent of the irradiated spores in this case could be attributed to an oxygen effect (Column D, Table 6) when survivors from the opened tube were compared with survivors from spores irradiated in vacuum. It is possible to compare experiments referred to the same initial population (Columns A and C, Table 6), and when this was done, the magnitude of the supposed oxygen effect could be readily evaluated. Irradiating the opened tube had the effect of reducing the viable spores from one-third to one-ninth the level in spores irradiated in vacuum.

In the next series of irradiation experiments we will attempt to prevent water absorption from the air during irradiation of spores at

atmospheric pressure. It will thus be possible to evaluate the influence of dry air on the irradiated spores.

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